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Original scientific paper

# BLOOD METABOLIC PROFILE OF THE DUBROVNIK SHEEP – CROATIAN ENDANGERED BREED –

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The Dubrovnik sheep is the most endangered Croatian indigenous sheep breed. The aim of the paper was to determine the blood metabolic profile and which blood metabolites can be used in nutritional status assessment of the Dubrovnik sheep. The investigation was conducted with 10 sheep of the Dubrovnik sheep breed at the average age of 3 years, during summer feeding season. The sheep were fed pasture and meadow hay. In the blood of the Dubrovnik sheep were determined the higher concentrations of sodium and chloride (175.40 and 128.60 mmol/l), and biochemical indicators (cholesterol – 2.34 mmol/l; triglycerides – 0.42 mmol/l; total proteins – 79.70 g/l and albumins – 32.40 g/l), as well as lower concentrations of inorganic phosphorus and iron (1.31 mmol/l and 23.52  $\mu$ mol/l) in comparison with the physiological values. Activities of enzymes and thyroid hormones in the blood of sheep ranged within physiological limits. Determined levels of most minerals (except inorganic phosphorus and iron) and biochemical indicators showed to an adequate supply of sheep with minerals, protein and energy and the possibility of lack of water during hot summer months. Blood metabolic profile is suggested to use as a reliable criterion in sheep nutritional status assessment.

Key words: Dubrovnik sheep; nutritional status; blood metabolites

## МЕТАБОЛИЧЕН ПРОФИЛ НА КРВТА НА ДУБРОВНИЧКАТА ОВЦА – ХРВАТСКА ЗАГРОЗЕНА РАСА –

Дубровничката овца е најзагрозена раса на овци во Република Хрватска. Целта на овој труд е да се утврди метаболичен профил на крвта, чиишто показатели можат да се користат во процената на хранидбениот статус на дубровничката овца. Истражувањето е изведувано во текот на целата летна хранидбена сезона со 10 овци од дубровничката раса. Овците беа со просечна возраст од 3 години. Престојуваа на паша, а по враќањето во штала консумираа ливадско сено. Во крвта на дубровничката овца се утврдени повисоки концентрации на Na и Cl (175,40 и 128,60 mmol/l), како и на некои биохемиски показатели (холестерол – 2,34 mmol/l; триглицериди – 0,42 mmol/l; вкупни протеини – 79,70 g/l и албумини – 32,40 g/l), и ниски концентрации на неоргански фосфор и железо (1,31 mmol/l и 23,52 mikromol/l) во споредба со нивните референтни вредности. Активноста на ензимите и хормоните на штитната жлезда во крвта на овците беше во рамките на физиолошките вредности. Утврдените концентрации на поголемиот број минерали (освен неорганскиот фосфор и железо) и биохемиските показатели во крвата укажуваат на адекватна снабденост на овците со минерали, протеини и енергија и мал недостиг на вода во текот на летните месеци. Утврдувањето на метаболичен профил на крвта може да се земе како квалитетен критериум во процената на хранидбениот статус на дубровничката овца.

Клучни зборови: дубровничка овца; хранидбен статус; метаболичен профил на крвта

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### 1. INTRODUCTION

The Dubrovnik sheep, known as the Dubrovnik ruda, is the most jeopardized Croatian indigenous sheep breed. By the CLC data (2008) total of 396 breeding-valuable sheep are raised in the Republic of Croatia in 22 sheep herds with very small number of sheep (18 animals/herd). In comparison with total breeding sheep in Croatia the Dubrovnik sheep participated with 1.16%. Preservation of genetic diversity of domestic animals is getting more important. Recognition, preservation and incentive of domestic animals specific genotypes breeding for certain breeding area and country are measures that should be taken from zoo-technique, ethical, cultural-scientific and other reasons aiming to preserve its own identity (Antunović et al., 2007). The Dubrovnik sheep-ruda represents the autochthonous Croatian breed created by disorganized merinization of domestic sheep populations in order to improve their production characteristics. It is reared in the narrow, but long coastal area of Dubrovnik, from Ston to Herceg Novi, encompassing the islands of Lopud, Šipan and Koločep, and less in the hinterland of Dubrovnik. The Dubrovnik sheep is of medium, but quite well built. It reared in smaller flocks primarily for meat and milk production and to a smaller extent wool production. Aiming to obtain more successful breeding of this sheep it is necessary to promote its technology. Prior to the required activities, the existing breeding quality should be determined. Due to feeding importance its status determination is one of the first directions which should be introduced aiming to assess present Dubrovnik sheep feeding. Disorder of nutrients metabolism often occurs if animals feeding is unbalanced or due to food nutrients lack or excess. Recently, blood metabolite profile has been increasingly used in sheep nutritional status estimation (Klinkon and Zadnik, 1997; Herdt et al., 2000; Van Saun, 2000; Antunović et al., 2002; Antunović et al. 2007).

The paper aims to determine blood metabolic profile and which blood metabolites can be used in nutritional status assessment of the Dubrovnik sheep.

### 2. MATERIAL AND METHODS

The investigation was conducted with 10 sheep of the Dubrovnik sheep breed at the average

age of 3 years during the summer feeding season. The sheep were fed pasture and meadow hay ad libitum.

The sheep blood was sampled (10 ml) from the jugular vein, after morning, in sterile vacuum tubes Venoject® (Sterile Terumo Europe, Leuven, Belgium), centrifuged for 10 minutes at 3000 revolutions and frozen. Content of minerals (Ca, P-inorganic, K, Na, Fe and Cl), biochemical indicators (glucose, urea, creatinine, bilirubin, total proteins, albumin, triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol) and enzyme activity (ALT-alanine aminotransferase, AST-aspartate aminotransferase, ALP-alkaline phosphatase, GGT-yglutamyl transferase and LDH-lactate dehydrogenase) were determined by the apparatus Olympus AU640 in the thawed off blood serum. The thyroid hormones  $(T_3 - triiodothyronine, and T_4$ thyroxin) sera concentrations were determined using the radio immunisation method on automatic immunoanalyser IMX-ABBOTT.

Statistical analysis of data was performed by the computer program *STATISTICA* (2008). Results were statistically evaluated using descriptive statistic.

#### 3. RESULTS AND DISCUSSION

In these investigations higher Na and Cl concentrations were determined in the blood of sheep as well as lower concentration of P-inorganic and Fe compared to the reference values according to Kaneko et al. (1997) (Table 1). Concentrations of Ca and K were determined on the upper limit of reference values (Kaneko et al., 1997). Antunović et al. (2008) found similar concentrations of Ca, P, K and Fe in the blood of the sheep Tsigai breed. The analysis of sheep supply with minerals shows that, due to food/blood mineral relation, animal's supply with P, K, Na, Zn and Se is likely to be predictable, although metabolism of other minerals is primarily dependent on homeostatic processes (Herdt et al. 2000). Changes in the concentration of minerals may be related to the nutrition of sheep. Wealth of pasture with Ca and K and the deficit in P and Fe (Underwood and Suttle, 2000) is known. Higher concentrations of Na and Cl in the blood of sheep can be connected with somewhat higher salt level in the sheep pasture due to winds and salt deposited from the sea. Similar results were stated by Baranowski et al. (2002) and Pastrana et al. (1991). The change in Na and Cl was due to water restriction during summer months because it causes hemoconcentration phenomena as results of a lower blood water level (Casamassima et al., 2008).

# Table 1

*Mineral concentration (mmol/l) in the blood serum of the Dubrovnik sheep* 

Indicators , mmol/l	$\overline{x}$	s	min–max	Standard error	Reference values*
Ca	3.01	0.09	2.92-3.14	0.04	2.80-3.20
Р	1.31	0.28	1.05-1.75	0.12	1.62-2.63
Κ	5.22	0.33	4.90-5.70	0.14	3.90-5.40
Na	175.40	4.04	171.00-181.00	1.81	139.00-152.00
Cl	128.60	5.77	119.00-133.00	2.58	95.00-103.00
Fe, µmol/l	23.52	4.22	18.00-29.00	1.89	29.70-39.70

\*Kaneko et al. (1997)

Animal energy status determination is very important in nutritional status assessment since it is reflected in animal productivity and health condition (Van Saun, 2000). Concentration of cholesterol, triglycerides, total protein and albumin were higher in comparison with reference values according to Kaneko et al. (1997). Assessment of the animal proteins supply is a rather complex procedure involving much more blood indicators. First of all it includes urea, total proteins, albumins and creatinine (Van Saun, 2000). Caldiera et al. (2005) reported that urea and protein concentrations are the most reliable indicators of the sheep protein status. In this investigation adequate concentration of urea and slightly higher concentration of cholesterol, triglycerides, total proteins, creatinine and albumins were determined compared with reference values according to Kaneko et al. (1997) (Table 2). These changes can be linked with a pasture rich on proteins, and water shortages during the summer months. Casamassima et al. (2008) have obtained higher levels of triglycerides, albumin, total protein and cholesterol in the experiment with sheep by water restriction. Other papers (Aganga et al., 1989; Jaber et al., 2004; Alamer, 2005) showed an increase in blood concentration of total proteins, urea, creatinine, cholesterol and electrolytes in different breeds of sheep subjected to water restriction.

# Table 2

Indicators, mmol/L	$\overline{x}$	S	min–max	Standard error	Reference values
Glucose	2.94	0.34	2.60 - 3.30	0.15	$2.78 - 4.44^{1}$
Urea	5.08	0.50	4.30 - 5.60	0.22	$2.86 - 7.14^1$
Cholesterol	2.34	0.32	1.90 - 2.70	0.14	$1.35 - 1.97^1$
Triglyceride	0.42	0.11	0.30 - 0.60	0.05	$0.0 - 0.2^{1}$
Creatinine, µmol/l	90.08	4.87	86.00 - 97.00	2.18	$50 - 109^2$
Total proteins, g/l	79.70	6.25	72.70 - 86.70	2.79	$60.00 - 79.00^{1}$
Albumine, g/l	32.40	0.57	31.70 - 32.80	0.19	$24 - 30^{1}$
HDL	1.51	0.22	1.26 - 1.86	0.10	_
LDL	0.66	0.17	0.50 - 0.90	0.08	_
Total bilirubin, µmol/l	2.60	1.52	0.01 - 4.00	0.68	$1.71 - 8.55^1$

Biochemical concentration in the blood serum of the Dubrovnik sheep

<sup>1</sup> - Kaneko et al. (1997), <sup>2</sup> - Baumgartner and Pernthaner (1994)

Thyroid gland hormone concentrations indicate energy supply, thus they can be taken as a good criterion in assessment of small ruminants energy status (Todini et al. 2007, Todini, 2007; Antunović et al., 2008). Activity of ALT, AST and GGT enzymes as well as urea concentrations can be recommended as a reliable liver status criterion (Van Saun, 2000 and 2007).

In these investigation activities of thyroid hormones and enzymes in the blood of the Du-

brovnik sheep were in physiological limits (Table 3).

## Table 3

Enzymes activity in the blood of the Dubrovnik sheep

Enzymes U/l	$\overline{x}$	s	min-max	Standard error	Reference values*
AST	97.00	15.03	83.00 - 122.00	6.72	60 - 280
ALT	17.80	4.55	13.00 - 25.00	2.03	6 - 20
ALP	132.20	84.35	45.00 - 236.00	37.72	68 - 387
GGT	52.60	24.75	9.00 - 69.00	11.08	20 - 52
LDH	363.20	34.98	314.00 - 397.00	15.65	238-440

\*Kaneko et al. (1997)

### Table 4

Activity of thyroid hormones and its ration in the blood of the Dubrovnik sheep

Indicators µmol/l	$\overline{x}$	S	min-max	Standard error	Reference values*
T <sub>3</sub>	1.11	0.15	0.92 - 1.27	0.07	_
$T_4$	68.39	11.01	51.48 - 81.98	4.92	54 - 110.7
$T_{3}/T_{4}$	0.017	0.003	0.01 - 0.02	0.001	_

\*Kaneko et al. (1997)

### 4. CONCLUSIONS

Determined levels of most minerals and biochemical indicators showed an adequate supply of sheep with minerals, protein and energy and the possibility lack of water during the hot summer months. Blood metabolic profiles are suggested to be used as a reliable criterion in the sheep nutritional status assessment.

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